activity regulated by Killer Immunoglobulin Receptor (KIR) genes may tip the balance towards either detrimental or beneficial outcomes. Both recipients and donors of 605 first allogeneic non-T depleted transplants were retrospectively KIR genotyped. In the non-HLA identical transplants, 92 inhibitory KIR ligand (iKIRL) mismatched cases displayed the worst five-year overall survival (OS, 20%) perhaps due to higher non-relapse mortality. In contrast, 75 transplants with only an HLA class-I antigen (Ag) incompatibility, but without an iKIRL incompatibility had significantly better OS (41%, $p<0.001$), which was similar to that (39%) of the Class I allele mismatched transplants, suggesting that incompatible iKIRLs exacerbated the effect of HLA Class I mismatches. In the HLA identical cases, the presence of both patient and donor 2DL/S2 resulted in a better OS (72% vs 58%, $p<0.01$) due to less relapse. Interestingly, the impact was concentrated in HLA-Cw iKIRL group 1 homozygotes. The absence of 2DL/S2 in the recipients decreased OS by 20% (72% vs 52%, $p<0.001$). The effect was not observed in the 10/10 (HLA-A, B, C, DRB1, DQB1) matched transplant cases, suggesting that, NK cell activity in HCT recipients, may be beneficial when the alloimmune response is less stimulated. Our study reflects the diversified role of NK cells in HCT, and suggests that KIR genotyping may be useful in clinical practice.

IMMUNE RECONSTITUTION

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MULTIVIRUS-SPECIFIC T CELL IMMUNOTHERAPY TO PREVENT OR TREAT INFECTIONS OF STEM CELL TRANSPLANT RECIPIENTS

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Viral infections cause morbidity and mortality in allogeneic HSCT recipients. To treat the underlying problem, namely lack of antigen-specific T cells, we and others have successfully generated and infused adoptive T-cell lines specific for EBV, CMV and Adenovirus. We have shown that as few as 2×10^5/kg trivirus-specific cytotoxic T lymphocytes (CTL) proliferated by several logs post-infusion and appeared to protect the recipients against all three viruses. Despite the encouraging clinical results, broader implementation of the approach is limited by (i) the usage of infectious virus material (EBV/Adv) and (ii) the prolonged culture (3 months for EBV-LCL production and CTL stimulation). Finally, (iii) "antigenic competition" between multiple viral antigens limits the extension to additional viruses. To overcome these limitations we have developed an approach to rapidly produce multivirus-specific CTL without using adenoviral vectors or EBV-LCL. Using nucleofection we consistently detected GFP transgene expression of ~39% of transfected DCs, without adversely affecting cell viability or DC maturation status. Coculture of p-Shuttle-pp65-GFP-transfected DCs with PBMCs from CMV seropositive individuals reactivated CMV-specific T-cells, which were detectable 9 days after stimulation, at higher frequencies (as measured by g-IFN ELIspot and Pentamer analysis (HLA-A2, NLV)) than lines generated using our standard protocol with Ad5f35pp65-transduced DCs as APCs. Importantly, this protocol could also be used to reactivate T cells against multiple viruses. Using a panel of p-Shuttle plasmids encoding LMP2 and BZLF1 (EBV), Large T (BK), and Hexon and Penton (Adv), we amplified CTLs from seropositive donors specific for the different viruses. Furthermore, we demonstrated that by pooling transfected DCs prior to PBMC stimulation, we could reproducibly generate multivirus-specific CTL lines, specific for all the stimulating antigens, without observing antigenic competition. To shorten the CTL production process, we established the selection of highly virus-specific T cells by IFN-g capture 24 hours after DC stimulation. In summary, we have established two GMP-applicable protocols for the rapid generation (<10 days) of CTL without using infectious material. In 10 days we can generate mono- or multivirus -specific CTL lines for prophylactic application. By performing IFN-g selection we can rapidly generate virus-specific CTLs for treatment of acute infection.

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DONOR LYMPHOCYTE INFUSIONS DEPLETED OF ALLOREACTIVE T CELLS DECREASE INFECTIONS WITHOUT CAUSING GVHD AFTER HAPLOTYPE MISMATCHED MYELOABLATIVE STEM CELL TRANSPLANTATION

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The ability to accelerate immune reconstitution following haplo-mismatched stem cell transplantion (SCT) would limit infections and disease relapse, and provide a unique opportunity to transplant the large number of patients who cannot find an HLA-matched donor. We present results of a Phase I clinical trial of haplo-mismatched allogeneic SCT supplemented with a donor lymphocyte infusion (DLI) photodynamically depleted of host-reactive T cells using dibromorhodamine as photosensitizer and visible light illumination (512 nm) (ATIR-PDT, Kiadis Pharma). Nineteen patients (11 M, 8 F) with high-risk hematologic malignancies (mostly refractory or relapsed acute myeloid leukemia (10) and myelodysplastic syndromes (4)) entered the trial. Median age at SCT was 54 years (range: 19–62). Increasing doses of PDT-treated donor cells (1×10^4 to 5.0×10^6 CD3+ cells/kg) were administered on day 34 ± 6 after transplant. Greater than 95% of anti-host cytotoxic T lymphocyte precursors (CTLp) were depleted from DLIs. All stem cell grafts underwent in vitro immunomagnetic T cell depletion using CD34+ positive cell selection (Miltenyi). The myeloablative regimen consisted of TBI (1200 cGy), thiotepa (5 mg/kg) and fludarabine (40 mg/m^2/day for 5 days) followed by infusion of CD3 depleted hematopoietic stem cell grafts. No GVHD prophylaxis was administered. All patients showed complete donor chimerism and durable hematologic engraftment. No patient developed acute GVHD (grade II-IV). Eight patients died: 4 of relapsed leukemia (3 AML; 1 ALL) and 4 of infections, all after day+300. In the first 7 patients who received 1.3×10^5 or less CD3+ cells/kg, 11 infectious episodes (4 lethal) occurred in these 7 pts (100% of pts). In sharp contrast, only 5 infectious episodes (none lethal) were observed in 3 (27%) of the following 11 patients receiving DLI with the highest doses of CD3+ cells (3.2×10^5 to 5.0×10^6 CD3+ cells/kg) ($p<0.05$). Chronic GVHD developed in 5 patients, mostly in those receiving higher T cell doses, and responded rapidly to steroid-based immunesuppression. The overall survival is 67% at 2 years (median follow-up: 10.5 mo). Our results indicate that the post-transplant infusion of an ATIR-PDT treated DLI is feasible, does not induce acute GVHD, and suggests a clinical benefit for patients receiving the highest DLI doses to accelerate T cell reconstitution.

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RAPID RECOVERY OF ABSOLUTE LYMPHOCYTE COUNTS (ALC) IS PREDICTIVE OF FAVORABLE OUTCOMES FOLLOWING UMBILICAL CORD BLOOD (UCB) TRANSPLANTATION: IMPACT OF THE USE OF TWO UCB UNITS

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After UCB transplantation, neutrophil recovery is markedly slower than other stem cell sources. The kinetics of lymphocyte recovery and their significance has not been extensively evaluated. We analyzed the maximum weekly ALC based on total white count and automated differential. To assess the impact of ALC on transplant outcomes, we analyzed 80 consecutive AML patients transplanted with UCB after a myeloablative conditioning from 2001–6. Six patients with graft failure or early death were excluded, resulting in 74 (92.5%) evaluable patients. The median age was 21.4 yrs (range 0.5–43.9). The majority of patients (85.1%) received cyclophosphamide (60mg/kg x 2), fludarabine (75mg/m^2)